Glia and neuron interactions: their role in synapse remodeling *in vivo*

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Recent advance in *in vivo* imaging techniques reveals the dynamic change in sensory and motor cortex following central and peripheral nerve damage. Functional changes in corresponding motor and sensory processing abilities are likely to be attributable to the changes in these neuronal circuits. The temporal and spatial limitations in the resolution of large scale imaging, such as MRI and NIRS, make these not ideal approaches to understand the underlying mechanisms at the neuronal circuit level. Two photon excitation of fluorescent molecules enabled high resolution observation of the fine structures and neuronal activity of cortical circuit plasticity *in vivo* in ischemic brain and after peripheral nerve injury (chronic pain). We focus here on the contribution of the glia cells to circuits remodeling.

For *in vivo* imaging, mice were anesthetized with either urethane (1.7 g/kg body weight, i.p.) and atropine (0.4 mg/kg body weight, i.p.), or with ketamine (0.13 mg/g) and xylazine (0.01 mg/g, i.p.). All procedures were approved by the Ethics Review Committee for Animal Experimentation of the National Institutes for Natural Sciences. At the penumbra area of ischemic mouse cortex, close to the ischemic core, there is massive remodeling of pre- and post-synaptic structures, being both generated and eliminated (synapse turnover). An enhanced rate of turnover was maintained for 1 month after ischemia. Microglia surveillance of the synaptic structures was greatly affected by ischemia. In healthy brain, the resting microglial processes made direct contacts onto neuronal pre- and postsynaptic structures with a very constant duration (5 min) and at a frequency of about once per hour. In contrast, the contact duration of microglia onto synapse following transient ischemia was markedly prolonged (1 hour), and these prolonged contacts were frequently followed by disappearance of presynaptic boutons. Thus, microglia *in vivo* survey the functional and pathophysiological status of synapses, with microglial-synapse contacts determining the subsequent fate of damaged synapses - to remain, or to be eliminated (Wake *et al.*, 2009).

Peripheral nerve injury triggers plastic changes along the somatosensory system that alter nociceptive signal processing, causing, for example, tactile allodynia (painful response to innocuous mechanical stimuli). The early injury-induced afferent barrage could drive alterations in the central neuronal circuits that contributes to chronic pain. Repeated two photon imaging revealed that spine turnover (loss and gain) in the S1 area corresponding to the injured paw markedly increased during an early developing phase of neuropathic pain, and was restored in the later phases of neuropathic pain. Preexisting stable spines were more prone to loss following injury, while spines that were generated after the injury tended to survive (Kim & Nabekura, 2011). Hence peripheral nerve injury induces rapid and selective remodeling of cortical synapses. Functional remodeling of both excitatory synaptic transmission (Eto *et al.*, 2011) and inhibitory transmission (Eto *et al.*, 2012) in Layer 2/3 of somatosensory cortex results in exaggerated neuronal responses to peripheral stimulation that contributes to allodynia. The activity of astrocytes was markedly enhanced during the early, but not during the later phases. Direct photo-activation of astrocytes accelerated local synapse remodeling. Thus, in peripheral nerve injury or inflammation, an initial transient and rapid activation of glia might contribute to synapse re-organization, resulting in the allodynia that characterizes the chronic pain behaviors.

Our studies reveal that the advances in imaging of fine structures in the living brain are contributing to better understand the synaptic and neuronal dynamics that contribute to brain function, and how these change in various pathophysiological conditions.

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